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Declaration under 37 CFR 1.132

Immunoassay's were originally described and developed using polyclonal antiserum produced by immunizing a species of animal (normally, a rabbit, goat, sheep, or horse) which differed from the source of the antigen one desired to produce an antibody against. Immunoassay's developed using polyclonals provided challenges in development due to sensitivity and specificity challenges as well as, frequent non-specific reactions, cross-reactivity with other related and unrelated antigens. Likewise, the large scale manufacture of products utilizing polyclonals suffered from a number of problems including; lot to lot variability, high backgrounds, non-specific binding, and unexpected cross-reactivity observed under actual use conditions in routine clinical laboratories.

In the mid 1970's, the problems observed with polyclonal antisera were addressed through the development of affinity purified reagents. These affinity purified reagents addressed a number of the issues observed with the polyclonal reagents, particularly cross reactivity, but they still consisted of a plurality of antibodies versus the target antigen which still left the affinity reagents susceptible to the performance problems of reproducibility and lot to lot variation.

With the advent of monoclonal technology, a researcher or development scientist finally had a tool that allowed them to develop experiments/products utilizing antibody reagents containing one species of antibody with documented performance characteristics. Thus, resulting in immunoassays where the developer/manufacturer had control over the nature and consistency of the reagent used in their assay.

The utilization of these monoclonal reagents revolutionized how immunoassay's were developed and produced essentially opening whole new product and assay areas, such as antigen detection, that were not possible due the problems associated with the use of multiple antibody or polyclonal antisera.

It might appear that if the use of one antibody clone to detect an antigen is effective, then a simple mixture of additional clones as suggested by Naritoku *et al*, should be more effective. Naritoku, W. Taylor, C. A Comparative Study of the use of Monoclonal and Polyclonal Antibodies Using Three Different Immunohistochemical Methods. J. Histochem. and Cytochem. Vol.30 1982 p253-60. Such is not the case. In addition to steric and pro-zonal hindrances, created by the number of antibodies in a given volume, there are variations in antibody affinities that dramatically affect assay results. A mixture of monoclonal antibodies might, therefore, behave like a polyclonal preparation, eliminating the advantages of using a monoclonal antibody.

Crowther teaches, in the case of polyclonal antibodies, higher affinity antibodies dominate specific interactions while excluding lower affinity antibodies. Crowther, J. The ELISA Guidebook, Vol. 149. p129 Humana Press. A few high affinity antibodies might, therefore exclude a number of low affinity antibodies and reduce the overall detection signal. At lower overall concentrations of polyclonal antibody preparations, more numerous low affinity antibodies dominate, and bring the sensitivity of the assay into question. Therefore, individual contributions of component antibodies need to be considered and adjusted. In the case of polyclonal mixtures, antibody concentrations can only be minimally adjusted, as a whole to establish new binding equilibria. Further optimization of individual clone concentration is prohibitive or impossible.

In the case of a monoclonal antibody, (first prepared by Kohler *et al* in 1975) only one antibody of a single type is present and its concentration can be finely adjusted, at the outset, without worry of disruption of other antibodies. Sevier *et al*, teach a monoclonal preparation can be affinity purified to yield a highly selective antibody, superior to a broadly reactive polyclonal preparation. Sevier, E. Monoclonal Antibodies in Clinical Immunology. Clin. Chem. 1981, p1797-1806. To reduce the broad reactivity of a polyclonal preparation, it has to be further adsorbed to cross reactive proteins, which does not totally remove "undesirable reactants" and also removes the "desirable reactants." To ensure specificities comparable to monoclonal antibodies Kierkegaard and Perry commercialized affinity purified polyclonal antibodies in the late 1970's, http://www.kpl.com/about/index.cfm?pID=7 but given the initial compositions and processing of the polyclonal antibodies it was impossible to maintain the batch-to-batch consistency of monoclonal antibody production.

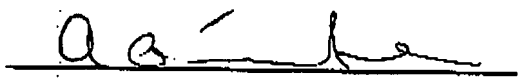
Robins teaches that when using monoclonal antibodies, stoichiomentry and therefore, antibody concentration is critical. Robins A. Immunochemical Protocols, Vol. 80. p337-46. Human Press.

At high antibody concentrations relative to the targeted epitope, monovalent binding predominates. Monovalent binding is much less stable than divalent binding and the "off-rate" of the antibody increases. While more antibody might initially bind it antigen, the bond is less stable. In samples that are not routinely washed of excess antibody, such as in flow cytometry, the correlation of labeling to antigen content becomes questionable.

A mixture of monoclonal antibodies is analogous to a polyclonal preparation. As a result, one must consider the specificity, kinetic effects and steric effects of one clone on another, and also consider the critical importance of concentration, taught by Robins, with regard to monoclonal antibodies. Simply creating a mixture of multiple monoclonal antibodies for an entire protein or protein isoforms is possible as Naritoku suggests. However, such a mixture produces a suboptimal and inaccurate detection system for a single epitope. It is crucial to consider the individual contributions of each component antibody and optimize the concentration of each antibody accordingly. At the same time one must ensure that the overall antibody concentration of the mixture does not perturb the more optimal divalent binding of each individual antibody. Therefore, the art teaches away from the proposition that, for any specific assay, making a mixture of monoclonals will result in a functional immunoassay.

I hold a Dr. P.H. from the University of Michigan and a BSc from Philadelphia College of Pharmacy and Science.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Drew O'Beirne
2212 E Pratt St
Baltimore, MD 21231